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European Community Research on Environmental Mutagenesis and Carcinogenesis

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Within the 12 Member States of the European Community (EC), environmental policy is now formulated primarily at Community level. As a result, the EC has important regulatory responsibilities for the protection of workers, consumers, and the general public from risks that may arise from environmental chemicals, foremost among them potential carcinogens and mutagens. An important part of EC environmental research and development is intended to provide a scientific basis for these regulations as well as increasing understanding of the basic mechanisms involved in environmental carcinogenesis and mutagenesis. This paper contains a brief introduction to EC environment policy and research, followed by an overview of EC chemicals control activities that are of particular relevance to the research and development program. Community-level research on environmental mutagenesis and carcinogenesis is then reviewed in some detail, including the achievements of recent projects, the scientific content of the current program, and perspectives for the future.

Introduction

The protection of the environment and the quality of life are of the highest political and public interest. The European Community's (EC) first Action Programme on the Environment was agreed on in 1973. With the signing of the European Single Act in 1987, the Community was given significant new responsibilities for environmental protection. It is fair to say that environmental policy and regulation is now primarily carried out at Community, rather than at Member State level. By now, there are more than 200 pieces of EC legislation on the environment, covering pollution of the atmosphere, water, and soil, waste management, safeguards in relation to chemicals and biotechnology, product standards, environmental impact assessments, and the protection of nature.

Over these last 20 years or so, there has been a discernible shift in EC environment policy away from reactive measures to combat acute environmental pollution problems toward increasingly broad-scale and preventive approaches. For example, when Community industrial, agricultural, transport, energy, and regional development policies are now formulated, their likely implications for the environment are assessed. The Community has also clearly underlined its commitment to environmentally sustainable development.

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European Community Research and Development on Environmental Protection

The Single European Act stipulates that: "in preparing its action relating to the environment, the Community shall take account of available scientific and technical data." EC-level research and development of the environment has the following broad objectives: *a*) to provide scientific and technical data that support the Community Environment Policy, *b*) to address longer-term environmental problems in support of preventive and anticipatory policies, *c*) to serve as an instrument for enhancing further, at Community level, the coordination of research activities in the environmental field.

EC environmental research is basically implemented by in-house research and development (R&D) or by shared-cost contract R&D. The former is carried out at the Joint Research Centre's Environment Institute in Ispra, Italy. R&D on environmental chemicals is an important part of the Institute's work, and also includes the operation of the Environmental Chemicals Data and Information Network.

Shared-cost contract R&D in the environment field is managed by the Environment Research Division of the Commission's Directorate General for Science, Research and Development (DG XII), and this is the subject of this article. The resources provided for EC contract R&D have increased substantially in recent years; this reflects the high priority given to environmental issues by Member

States, and recognition of the crucial role of research and development in tackling these issues. The current Environment R&D program (STEP and EPOCH, 1989–1992) have an allocation of 115 MECU (1 MECU = 1 million ECU. 1 ECU is approximately 1.35 in U.S. dollars); the new EC Environment R&D Programme (1991–1994) has a budget of 260 MECU. Contracts are awarded on the basis of competitive research applications in response to a public call for proposals. Projects must be transnational, with partners from at least 2 EC Member States. The average project has about 5 partners, although in some cases many more are involved.

In addition to the twelve EC Member States, Sweden, Norway, Finland, and Austria participate fully in this Programme. Institutes from other European countries can participate on a project-by-project basis; however, at present, their participation cannot be supported by Community funds. There is intensive competition for EC research funding; on average only about one in eight proposals can be financed.

EC environmental R&D programs cover the whole spectrum of environmental issues, including mechanisms and impacts of global change, in particular climate change and depletion of stratospheric ozone; clean technologies, waste management, industrial accidents, protection of cultural items; socioeconomic environmental research; and natural and technological risks, including research on genetic effects of chemicals and on biomonitoring of populations exposed to genotoxic substances.

Control of Environmental Chemicals in the European Community

As mentioned in the previous section, a primary objective of EC environmental R&D is to provide a sound scientific basis for EC environmental policy. Research on genetic effects of chemicals and on biomonitoring techniques for genotoxic substances is intended to support this policy, particularly in relation to the control of chemical substances.

The overall aim of chemical control by the EC has been to develop comprehensive legislation to harmonize procedures and standards in such a way as to allow free circulation of goods and encourage industrial competitivity and technological development while, at the same time, ensuring a high level of protection for man and for the environment. Several complementary approaches have been adopted. These include directives on the classification and labeling of dangerous chemicals, the systematic evaluation of existing chemicals, protection of animals used in testing, protection of workers, etc.

Classification and Labeling of New Chemicals

Classification and labeling was the first EC chemicals directive, adopted in June 1967, and amended since seven times. The Sixth Amendment, agreed on in 1979, carried the most significant change, requiring mandatory notification of new chemical substances 45 days before they are placed on the market. It was one of the first truly "preventive" rather than "curative" EC environmental actions. The evaluation required for notification is carried out on the basis of data presented in the notification dossier, which consists of four elements: *a*) a technical dossier supplying the information necessary for evaluating the foreseeable risks, whether immediate or delayed, that the substance may entail for man and the environment, *b*) a declaration concerning the unfavorable effects of the substance in terms of the various uses envisaged, *c*) the proposed classification and labeling of the substance in accordance with the directive, *d*) proposals for any recommended precautions relating to the safe use of the substance.

The technical dossier is defined in Annex VII of the 1979 Directive and includes information about the identity, use, quantities, and disposal of the substance, as well as the basic physicochemical, toxicological, and ecotoxicological test results. For genotoxicity testing, the requirements are indicated in Table 1. Requirements vary according to the quantities marketed per year. To ensure harmonization at the testing level, Annex V of the Directive clearly specifies the test methods to be used.

Systematic Evaluation of Existing Chemicals

The EC had been notified of around 600 new chemical substances (by March 1991). However, there are over 100,000 chemicals that were placed on the market before the notification scheme came into force in September 1981—these are the "existing chemicals." The European Inventory of Existing Commercial Chemical Substances (EINECS) includes, for each substance, its EINECS number, chemical name, its molecular formula, and its CAS number. On the basis of current knowledge, production quantites, structure–activity relationship approaches, etc., a list of about 25 substances is to be established each year for priority attention. A risk assessment will be carried out on these chemicals and the possible needs for regulatory action considered.

Table 1. EC Council Directive 79/831/EEC (Sixth Amendment).

	Testing for genetic toxicity
Level 0	Technical dossier (Annex VII)
1–10 tons/year	(Base set)
Two tests	Gene mutation test in *S. typhimurium* or *E. coli*
	Chromosomal aberration test in mammalian cells (*in vitro*) or the micronucleus or the metaphase analysis of bone marrow cells (*in vivo*)
Level 1	
10–100 tons/year	Optional
>100 tons/year	Obligatory
Four tests	Two tests of the base set plus
	Gene mutation test in eukaryotic cells
	Chromosomal aberration test (*in vivo* or *in vitro*)
Level 2	
>1,000 tons/year	Long-term carcinogenicity

Protection of Workers

Community directives concerning protection against the effects of chemicals in the workplace are of general application across the spectrum of industry from production to placement on the market to final disposal. These actions are based primarily on two framework directives. Three types of control may be introduced: *a*) for highly dangerous agents of the type industrial society could and should dispense with, a ban on production and use (e.g., certain amines); *b*) for other highly dangerous agents (e.g., carcinogens) that cannot reasonably be banned, highly effective worker protection systems using, wherever possible, of closed systems; *c*) for other dangerous agents, a monitoring system based, *inter alia*, on exposure limit values.

Other Relevant Measures

There are two other EC directives that are of particular relevance to EC R&D on genetic effects of chemicals. One is the "Seveso Directive" of 1982 concerning major industrial hazards of certain industrial activities. The directive contains agreed-on minimum requirements on preventive measures at the stage of plant or process design; installation of control and safety measures as well as preparation of emergency plans; and control of major industrial accidents (if they occur) including sharing information concerning the causes, emergency, medium, and long-term measures adopted, and the extent of damage.

Finally, the Community has responded to public concern about the use of animals in testing: in 1986 a Directive was adopted concerning the "approximation of laws, regulations, etc., regarding the protection of animals used for experimental and other scientific purposes." In recognition of the needs for scientific progress in this field, in 1991, the Commission has established a European Centre for the Validation of Alternative Methods, to be located at the EC's Joint Research Centre in Ispra, Italy.

During the next 10 years, the EC will continue to improve control of environmental chemicals. Some significant, planned initiatives are outlined in Table 2.

Scientific Content of EC R&D on Genetic Toxicity

Cooperative R&D on the genetic effects of environmental chemicals has been a feature of EC environmental R&D for more than 15 years. During earlier programs, numerous test systems using bacteria, fungi, cultured cells, Drosophila, and whole animals have been studied, involving comparative assessments by the use of reference mutagens. The results obtained were of direct application in the definition of test protocols incorporated in the Annexes to the Sixth Amendment and in the overall database on the validity of short-term tests. The following sections contain an overview of some past R&D programs, an outline of current work, and discussions of future perspectives.

Table 2. Planned initiatives of the European Community.

Data collection	Effective notification procedure for all chemicals
Hazard identification	Maintenance/improvement of existing classification criteria
Risk assessment	Common principles for assessments
	Assessment of high production volume chemicals
	Assessment of active substances in nonagricultural pesticides
Risk management	Strengthens links between classification and control measures
Risk reduction	Risk reduction programs for selected priority chemicals.

Some Achievements of the Previous R&D Programs

In this section, some of the main achievements of the two previous R&D programs in this field are reviewed. These were the Third Programme (1981–1985) and the Fourth Programme (1986–1990). Detailed scientific reviews of the former have been published (*1,2*).

The overall scientific output of the Third R&D Programme was examined by a bibliographic survey, which was commissioned by an independent evaluation panel. This survey found that in all areas covered by the R&D program on genetic effects of environmental chemicals, the number of published papers in quality peer-reviewed journals, as a proportion of total European and world outputs, was far in excess of its relative size in terms of financial and manpower resources.

Correlations between Mutagenicity Tests and Other Assays for Carcinogenic Potential. The observed correlation between the ability of a chemical to induce mutation in various test systems and its carcinogenic potential has provided much of the incentive for test system development. The Third Programme focused on cell transformation methods as potential assays for carcinogens. Results indicated that transformation assays provide models for the progressive stages of tumour induction and development. This was further developed during the Fourth Programme as assays for nongenotoxic or epigenetic carcinogens.

Host-mediated Assay. The host-mediated assay involves use of indicator bacteria injected into a small rodent. Indicator species are then used to determine mutagenic effects of a chemical given to rodents. The project identified many variables in this assay such as the effects of rodent strain, age of rodent, and effects of diet. This assay has to some extent been bypassed by newer technologies such as measurement of unscheduled DNA synthesis (UDS) in specific tissues, transgenic animals, and direct measurement of DNA base changes.

UDS in Cultured Mammalian Cells. Here, the principal result of the Third Programme was an understanding of the importance of the measurement of DNA synthesis in the nucleus and in the cytoplasm. Further development of this assay for use in the liver of the rat has provided an important regulatory method.

Metabolic Activation. In the Third Programme, variables examined included inducers of P450, growth conditions to improve activation potential by yeasts, rodent strain differences, and constituents of S-9 mix. In the Fourth Programme, this project was broadened into the development and validation of metabolic activation systems in mutagenicity testing that are not derived from animals.

Mutagenicity of Aerosol Extracts. The aim of this work was to produce a mutagenic fingerprint of aerosol samples, to characterize the potential genotoxicity of each component, and to assess the overall mutagenicity of the mixture. The studies indicated that the samples contain genotoxic chemicals. However, the work was less successful in identifying the specific constituents of the genotoxic extracts and the overall mutagenic profile of the samples.

Transplacental Genotoxicity. The mammalian transplacental assay system was developed to the stage where it can be used to investigate differential sensitivity of the fetus to specific environmental chemicals. The sensitivity of specific stages of fetal development may be assessed, and the effects of modifiers of metabolic activity can be determined.

Aneuploidy. The Third Programme demonstrated that a large number of chemicals induce aneuploidy in fungi. At the same time, several questions arose in relation to the relevance of this system to man—for example, whether fungi can be used to screen aneugens and whether a suitable regulatory assay can be developed. In continuing this work, the Fourth Programme showed that fungi are not suitable for screening, and further evidence was obtained of the complex mechanisms by which chemicals induce aneuploidy, as well as confirmation that aneuploidy is important in tumor development.

Development and Validation of Animal Tests. Animal tests are essential for labeling mutagens. Comparisons of several tests were performed, including specific locus, bone marrow cytogenetics, specific-tissue UDS, mouse spot test (in embryos), dominant lethality, germ cell cytogenetics, and tumor promotion. This work underlined the need to apply new technologies to improve resolution and quality and to reduce animal usage.

Drosophila. Assay systems using the fruit fly, *Drosophila melanogaster*, allow the detection of a wider range of genetic end points, study of somatic and germ cells, and study of the biotransformation of many chemicals. The studies have demonstrated the value of this system for the genotoxic assessment of environmental chemicals. A notable achievement was the development of the SMART somatic assay, which may provide information for specific purposes.

Development of New Technologies. The development and application of new technologies has been an important feature of the programs. Examples include *a*) development and use of specific monoclonal antibodies; *b*) molecular probes for detecting specific DNA sequences; *c*) new molecular approaches to measure DNA base changes in exposed cells *in vitro* and *in vivo*.

Molecular Dosimetry. The purpose of the molecular dosimetry project is to study the possibilities of using DNA adduct formation as a parameter for comparative studies. Model chemicals used were a series of ethylating agents. Genetic effects in different assay systems (*E. coli*, Drosophilia, cultured mammalian cells, mouse germ cells) were compared and DNA adduct frequencies were determined.

During the first period (1982–1985), the technology was developed and the initial experiments were carried out, showing that in fast-growing cells the induced mutant frequencies were the same for all ethylating agents when compared at equal frequencies of O^6-ethylguanine. This suggests that most of the genetic effect induced by the ethylating agents is caused by the DNA adduct O^6-ethylguanine. This observation was followed up in the next program (1986–1990) by carrying out experiments using methylating agents. The data in germ cells of mice show that there is a strong specificity of mutation induction for specific stages of germ cells. The data also suggest that in germ cells the O^6-alkylguanine adduct is not the mutagenic lesion. More recently, molecular techniques have been developed that allowed DNA sequencing of induced mutations. This approach showed that in growing cells the majority of the mutants induced by ethylating agents are at GC base pairs, whereas in mouse germ cells all mutants analyzed are at AT base pairs.

Biomonitoring Human Populations Exposed to Environmental Genotoxic Chemicals. This project, launched by the Commission in 1988, is intended to assist the development of population monitoring systems designed to quantify the exposure to potential mutagenic chemicals in the environment and to detect possible early effects. During the last decade, great progress has been made in methodologies for assessing exposure to and biological effects of genotoxic chemicals. New methods for the identification of DNA damage *in vivo* offer the possibility to quantify individual exposure.

In the first phase, the Biomonitoring Project consisted of the development, comparison, and intercalibration of advanced techniques for the quantification of a molecular (target) dose and its comparison to external exposure. The approach is based on the measurement of DNA and protein adducts in relation to selected genetic end points.

Eleven laboratories from eight European countries cooperated closely on various aspects of methodological development, for *a*) the identification and measurement of DNA adducts by ^{32}P-postlabeling method, immunological assays, and advanced mass spectrometry technique; *b*) the detection of hemoglobin and plasma protein adducts by gas chromatography-mass spectrometry; *c*) the detection of chromosomal aberrations, sister chromatid exchanges, and micronuclei in peripheral lymphocytes; *d*) the quantitative determination of *hprt* mutant frequency in lymphocytes and mutations in hemoglobin gene. A detailed review of this coordinated project has recently been published (*3*).

The Current R&D Program

Work within the current program began in the latter part of 1991. There are 11 coordinated projects, involving

about 60 research institutions in 14 European countries. A brief synopsis of each coordinated project is provided below.

Detection and Evaluation of Aneugenic Chemicals. The aim of this project is to increase understanding of the effects of aneugenic chemicals in cultured mammalian cells, rodent bone marrow, and rodent germ cells and, on this basis, develop experimental systems capable of detecting and assessing environmental aneugens.

Nongenotoxic Carcinogens: Development of Detection Methods Based on Mechanisms. This project will investigate to what extent fibroblast transformation systems and inhibition of gap junctional intercellular communication in established cell lines can detect nongenotoxic carcinogens. The possibility of using other proposed mechanisms of nongenotoxic carcinogenesis to develop as assays will also be evaluated.

Development and Validation of Metabolic Activation Systems for Mutagenicity Testing Not Using Vertebrate Animals. This project aims to develop reliable *in vitro* systems to detect mutagenicity of indirectly acting mutagens/carcinogens. The work will include validation of cell lines capable of metabolizing mutagens (e.g., Hep G2, CHEL, V79/Cyt450, PHH); preparation of microsomal extracts of the above to act as exogenous metabolic activation systems; validation of plant activation systems, as well as hemoglobin and biomimetric-based metabolic systems.

Detection of Germ Cell Mutagens. The goal of this project is to improve recognition of germ cell mutagens in chemical substances and in the environment. Tests such as the mouse specific locus, heritable translocation assay, and dominant lethal are applied to identify germ cell mutagens. New methodologies such as spermatid micronucleus test and flow cytometry of sperm-DNA are validated against data from the former. A database on germ cell mutagenecity will also be established.

Somatic Recombination, Gene Amplification, and Cancer. Genetic recombination, gene amplification, and transposition may be of importance in tumor formation. This project will provide information on the occurrence of chemicals that may act as recombinogenic agents and inducers of recombination-related end points such as transpositions and gene amplification. The data obtained may also reveal systematic relationships between chemical structure and recombinogenic activity.

Development of New Methods for Detection of Genetic Alterations in Man. This project is intended to develop molecular methods that can detect mutations in any cell or organ of exposed individuals or experimental animals. The techniques should identify alterations at any chosen restriction site in a gene of interest. The specific objective of this project is to solve the main technical problems and to validate the approach in a limited number of test systems using different organisms.

Implications of Structure–Activity Relationships for Cross-Species Extrapolation in Mutagenesis and Carcinogenesis. The overall objective of this project is to develop risk assessment procedures for groups of structurally related chemicals. In this project, three groups of chemicals are to be analyzed; alkylating agents, cross-linking agents, and *N*-substituted aryl compounds. The work will attempt to elucidate correlations between chemical reaction parameters, reactivity with DNA, the types of genetic damage produced, and the position of each chemical on the "relative carcinogenic potency" scale.

Molecular Dosimetry of Chemical Mutagens. The aim of this project is to continue investigations of whether the frequency of DNA adducts in the target cells or target tissues can function as a measure of exposure on the basis of which the magnitude of genetic effects in different assay systems can be interrelated. In this project, DNA adduct frequency as a function of the exposure concentration of a series of mutagens will be determined in a number of organisms in which the induction of genetic effects is also measured. Analysis of the persistence of the DNA adducts and molecular analysis of the genetic effects at the DNA level will allow determination of the type of DNA adducts responsible for the observed genetic changes.

Biomonitoring Human Exposure to Environmental Genotoxic Chemicals. The overall objective of this project is to determine the additional genetic burden carried by individuals exposed to urban pollution. Samples will be collected from populations in urban locations. Physicochemical methods will be developed to assay adducts formed by aromatic compounds, low molecular weight alkylating agents, and radical DNA-damaging agents. Mutation and cytogenetic analysis will also be performed. Results will be compared with those from populations in industrial surroundings (positive control) and in rural locations (negative control). The biomonitoring methods will be compared for their relative sensitivity and applicability for exposure monitoring.

Biomonitoring Populations Exposed to Pesticides. Biomonitoring methods will be applied to evaluate professional exposure to a range of commonly used pesticides in parts of Italy, Spain, and Finland. Biological assays to be used include chromosome aberrations; sister chromatid exchanges; micronuclei; poly(ADP-ribose)polymerase; urine analyses; and cholinesterase. Chemical methods include DNA adducts; hemoglobin–amino acid adducts; and plasma protein adducts.

Assessment of Human Exposures to Butadiene as a Model for Risk Estimation of Petrochemical Emissions. Butadiene is an important industrial chemical and a frequent contaminant in traffic exhausts, ambient air, drinking water, and tobacco smoke. This project will include comparisons between environmental and biological monitoring methods, between cytogenetic effects and macromolecular binding, and between adduct levels of occupationally and environmentally exposed individuals. This work will entail development and validation of biomonitoring techniques and hazard estimation approaches for human cancer.

Supporting Activities

The significant EC research effort is supported by various activities intended to enhance its efficiency, to

maximize the added value of international cooperation, to assist dissemination of results, and to increase the relevance of the work to environmental and health protection policies. The scientists participating in this program meet, at least once each year, in contact group meetings. Here, progress is discussed, future directions agreed on, and new collaborative activities initiated. Each such meeting is, in turn, hosted by a different participating institution.

In some cases, the Commission participates in the organization of international workshops, symposia, etc. In this particular research field, organizational and financial support has been provided for recent annual meetings of the EEMS (European Environment Mutagen Society) and for a number of meetings hosted by IARC (International Agency for Research on Cancer) and ICPEMC (International Commission for Protection Against Environmental Mutagens and Carcinogens).

Finally, following the invitation of the OECD Chemicals Division, the Environment Research Programme acted as "lead country" in preparing the detailed review paper to revise OECD Guidelines for Genetic Toxicity Testing. This task was entrusted to three participants in the program (A. Carere, G. Mohn, J. Parry), who prepared the detailed reveiw paper in close consultation with other scientists in the EC program.

Future Perspectives

Within the new EC Environment R&D Programme (1991–1994), work in this area will continue to provide direct support for Community policies related to the screening, health risk assessment, and control of environmental chemicals. This includes short-term support through improvement of predictive testing of specific end points related particularly to carcinogenic and mutagenic risk and long-term research to elucidate the basic mechanisms of environmental carcinogenesis and mutagenesis. There are two main research themes, as discussed below.

Further Development of Genetic Toxicity Testing. Research will include further refinement and, where appropriate, validation of tests for established genetic end points, which are suitable for incorporation into existing regulatory protocols, as well as development of novel approaches and techniques to increase coverage of end points that may be associated with genetic disease and cancer. Particular emphasis is given to innovations that allow reduction in the use of animals in testing without detriment to predictive capability.

Relating Genetic Toxicity to Cancer and Heritable Mutations. In addition to improving the scientific and technical basis of regulatory schemes for genetic effects of chemicals, it is also intended to improve our understanding of the mechanisms by which exogenous agents may be involved in the etiology of cancer and mutations in man. This will facilitate prevention and risk assessment and lead to significantly more effective and efficient chemicals testing strategies.

Application to Urgent Environmental Problems

The research undertaken in this R&D Programme clearly requires highly advanced manpower and technical resources. However, as well as providing both immediate and longer-term support for European Community environmental and health policies, it is intended that the work should be of immediate relevance to urgent environmental health problems. For example, there may be increased hazards associated with elevated levels of potentially mutagenic or carcinogenic pollutants in various parts of Eastern and Central Europe. The European Community is actively assisting these countries to manage problems of this kind, and this assistance makes appropriate use of the scientific and technical capabilities developed within the EC Environment R&D Programme. This type of approach is also being established with Mediterranean countries, within the framework of the Community's International Scientific Cooperation activities.

The views expressed in this paper are the author's and not necessarily those of the Commission of the European Communities.

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